

Polymorphisms in Protease and Reverse Transcriptase in Treatment Naïve Individuals Infected with HIV-1 Subtype C in Southern Africa

Abstract

Understanding the sequence diversity of protease (Pr) and reverse transcriptase (RT) in all HIV-1 subtypes is crucial to rational design and assessment of drug therapy in developing countries. This study examined the Pr and RT sequence in 34 therapy-naïve individuals participating in the southern Africa (SA) HIVNET 028 study in Malawi, Zambia, Zimbabwe, and South Africa. All of the samples were HIV-1 subtype C. No polymorphisms that would be called major resistance polymorphisms in HIV-1 subtype B were observed in either the Pr or RT regions of these subtype C samples. Polymorphisms potentially relevant to resistance that occurred at relatively high frequency were: K20R, M36L, and I93L in the Pr, and R211K in the RT. Other polymorphisms potentially relevant to resistance were seen at lower frequencies, as were abundant polymorphisms not known to be related to drug resistance. These polymorphisms need to be considered when designing and assessing therapy in target populations.

Introduction

The genetic diversity of human immunodeficiency virus type 1 (HIV-1) has given rise to several subtypes and recombinant forms with uneven geographical distribution (1). HIV-1 subtype B is the most common subtype in the United States and Western Europe and most information on therapeutic efficacy and the development of drug resistance polymorphisms is based upon subtype B. The relevance of this information for patients infected with non-subtype B isolates has not been adequately studied. Baseline polymorphisms in protease (Pr) and reverse transcriptase (RT) genes linked to resistance to antiretroviral drugs in treatment-naïve patients have been reported in non-B subtypes by some studies (2,3). These polymorphisms do not directly confer high level resistance to an antiretroviral drug by themselves, but occur in the presence of other polymorphisms (4), and therefore may affect the efficacy of antiretroviral therapy and the paths to the development of resistance in patients with non-B subtypes (3,5).

This study analyzed the protease (Pr) and RT sequence of HIV-1 subtype C from southern African patients who were antiretroviral naïve in order to determine whether there was any evidence of pre-existing resistance polymorphisms in this population, and the frequency of polymorphisms that are associated with drug resistance. The frequency of these polymorphisms were compared with those of drug-naïve HIV-1 subtype B.

Methods

This study examined the Pr and RT sequence in 34 therapy-naïve individuals participating in the HIVNET 028 study in Malawi, Zambia, Zimbabwe, and South Africa. The *pol* sequence was determined from frozen plasma samples using the ViroSeq Genotyping System (Applied Biosystems, Foster City, CA) and DNA sequencing on a 377. Analysis of the sequences was done using Sequencher and ClustalX for alignment, the National Institutes of Health's Genotyping Tool (<http://www.ncbi.nlm.nih.gov/retroviruses/subtype/subtype.html>), and Stanford University's Sequence Analysis Program (<http://hivdb.stanford.edu/pages/seqAnalysis.html>) websites for classification, and the Los Alamos National Laboratory Antiviral Drug Resistance Analysis tool (<http://www.hiv.lanl.gov/content/hiv-db/ADRA/adra.html>) and Stanford University websites for base frequency analysis.

Results

Sequencing was successful on 34 of 40 samples on which sequencing was attempted. All of the samples were HIV-1 subtype C. No polymorphisms that would be called major resistance mutations in HIV-1 subtype B were observed in either the Pr or RT regions. Treatment-naïve subtype C infected individuals from SA had 10 naturally occurring polymorphisms in Pr and 8 in RT that have been identified as minor drug resistance mutations in HIV-1 subtype B, based on the databases from Applied Biosystems, Los Alamos National Laboratory and Stanford University. Polymorphisms potentially relevant to resistance are compared with frequencies of HIV-1 subtype B in the Stanford database (<http://hivdb.stanford.edu>).

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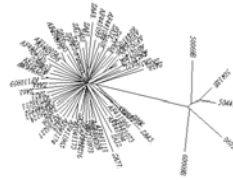


Figure 1: Phylogenetic tree analysis of our samples cluster together with 35 subtype C reference sequences obtained from the Los Alamos National Laboratory database and not with subtype B samples sequenced in our laboratory. Subtyping of our samples was based on National Institute of Health and Stanford University HIV-1 subtype C databases.

Polymorphisms in the Pr region of Southern Africa samples

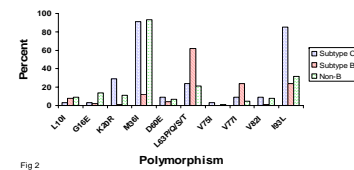


Fig 2

Figure 2: Proportion of resistance associated polymorphisms in the protease region of southern Africa samples compared with subtype B and non-B from Stanford University database. Polymorphisms K20R, M36L, I93L in the Pr occurred in at least 10 of 32 samples, and were comparatively less frequent in drug-naïve subtype B isolates with sample range of 645 - 852. Polymorphisms L63P/Q/S/T was found to occur more than two times in naïve subtype B than in naïve subtype C samples.

Polymorphisms in the RT region of Southern Africa samples

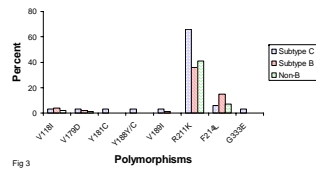


Fig 3

Figure 3: Proportion of resistance associated polymorphisms in the reverse transcriptase region of Southern Africa samples compared with subtype B and non-B data from Stanford University database. Minor resistance polymorphisms are rare in the RT. Polymorphisms V118I, V179D, Y181C, Y188Y/C, V189I, and G333E were found in only one sample each, while P24L was in 2 out of 32 samples. R211K mutation said to facilitate resistance to nucleoside reverse transcriptase inhibitors (NRTI) was very frequent, 66% of 32 samples and 36% of 309 samples in naïve subtype C and B respectively.

Enzyme	Mutation	% subtype C (Current study)	% subtype B (Stanford database)
Protease	L89I/M	97	0
	E36A	72	0
Reverse transcriptase	T39E/D	93	0
	K173A/T	100	0
	V245Q	76	0

Table 1. Proportion of polymorphisms with high frequency in SA samples but not recorded in subtype B data from Stanford University database. Five codons, 1 in Pr and 4 in the RT, have a high frequency of polymorphisms in drug-naïve subtype C, sample size 32, and are not recorded in drug-naïve subtype B Stanford University database with sample size range of 159 - 852. These polymorphisms are not known to be part of the subtype C specific cluster of amino acids, ERKM and KVEQ with positions 35, 57, 69, and 89 on Pr region and 65, 106, 138, and 161 on RT region respectively.

High frequency Polymorphisms in Protease region

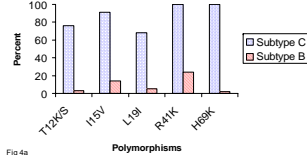


Fig 4a

Figure 4a: Proportion of polymorphisms with high frequency in SA samples but rare in subtype B isolates data from Stanford University database. Five codons in the Pr region in which the frequency of polymorphisms were at least four times higher in subtype C with sample size, 34, than in subtype B drug-naïve isolates with sample size range of 97-852. Amino acid K at position 69 is a subtype C cluster specific amino acid

High frequency Polymorphisms in Reverse Transcriptase

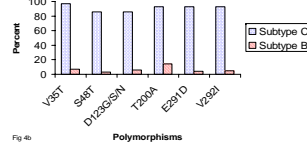


Fig 4b

Figure 4b: Proportion of polymorphisms with high frequency in SA samples but rare in subtype B isolates data from Stanford University database. Six codons in RT region in which the frequency of polymorphisms were at least four times higher in subtype C with sample size of 32 than in subtype B drug-naïve isolates with sample size range of 97-852 the Stanford database

Frequency of polymorphisms per given sample

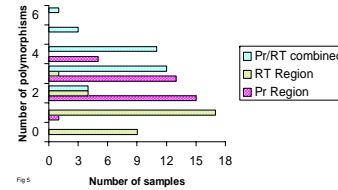


Fig 5

Figure 5: The number of polymorphisms in the protease (Pr), reverse transcriptase (RT) and combined Pr and RT regions in any given sample. Combined Pr and RT showed 3 polymorphisms per sample to be the most frequent at 39%. Polymorphisms were more common in the Pr as 97% (33/34) of the samples had at least 2 polymorphisms per sample. In the RT, only 16% of the samples had more than one mutation per sample. All the samples with both Pr and RT regions analyzed had at least 2 polymorphisms per sample. Of the 13% of the samples with no less than 5 polymorphisms each, one sample had pre-exposure resistance to Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) as determined by virtual phenotyping.

Figure 6: Alignment of 20 South Africa (SA) consensus and the reverse transcriptase of the protease region (Pr). Red indicates positions shared by the SA samples and shared by 100% naive and protease are shared by a number other a period (.) - under the number of that position aligns with the reference strain.

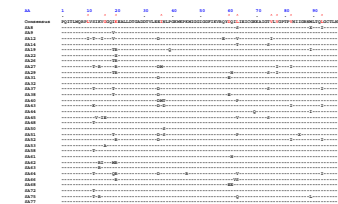
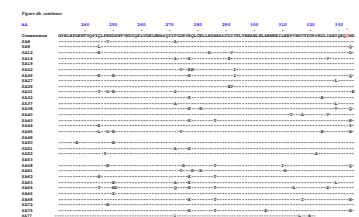
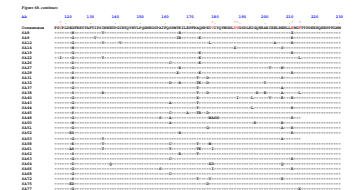
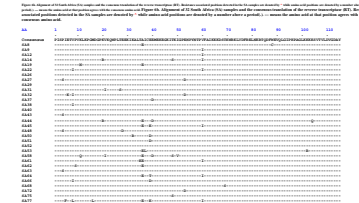


Figure 6: Alignment of southern Africa samples and consensus of *pol* translation. Fig 6a shows minor drug resistant polymorphisms distribution in the protease region of our samples. Fig 6b shows RT polymorphisms in our samples which were not as frequent as in the Pr region.

Three minor drug resistant polymorphisms, L10I, G16E, and V75I, in the Pr region and 6 polymorphisms, V118I, V179D, Y181C, Y188Y/C, V189I, and G333E, in the RT were found in a few samples but not in the reference samples. Sequence alignment consensus of 35 subtype C reference isolates from Botswana (21), India (6), Tanzania (1), South Africa (6), and Zambia (1) obtained from Los Alamos National Laboratory database matched perfectly with our southern Africa samples consensus alignment.



Conclusions

There were no natural major resistance mutations in either the protease or reverse transcriptase in any of the DNA sequences from treatment naïve individuals infected with HIV-1 subtype C in southern Africa. Differences in the frequency of accessory resistance-associated polymorphisms did occur, however, and need to be considered in countries with high prevalence of subtype C when designing and assessing therapy in target populations.

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